BINOD BIHARI MAHTO KOYALANCHAL UNIVERSIRTY

SYNOPSIS ON

"STUDIES ON THE IMPACT OF COAL WASHERIES ON THE PHYSICO-CHEMICAL PARAMETERS AND ITS EFFECT ON THE HISTOPATHOLOGY OF LIVER OF FISH SPECIES OF DAMODAR RIVER OF BOKARO DISTRICT OF JHARKHAND"

For

DOCTOR OF PHILOSOPHY

IN

FACULTY OF SCIENCE (ZOOLOGY)

UNDER THE SUPERVISION OF

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1. State of the art:

Damodar river is one of the prominent tributaries of our holy river Ganga. It originates from the Khamapet hill, Palamau district of Chotanagpur plateau of Jharkhand. It travels about 592 km in the eastern part of India and confluences to the river Hooghly at Kolkata. During its course, the river flows through large cities like Hazaribagh, Bokaro, Dhanbad, Asansol, Durgapur, Howrah etc. Saha *et al*.2013 have reported that in some of the local languages of Jharkhand. Damodar river is called "Damuda", damu means sacred and da means water. [1]. The river Damodar flows through the mineral rich areas that produce about 37.2%coal, 35%bauxite and 43% mica in the country. Moreover, the river is the main source of water to the industries that produce 310 million tons of coal, 80 million tons of steel and 2000 MW of thermal and hydel power, which together contribute substantially to the country's economy. (source-www.cifri.res.in, bulletins, The river Damodar and its environment)

Industrial Agglomeration:

There are altogether 131 major industrial establishments including the coal mines along the river side which can be broadly classified as: --

A) Coal mines

B) Coal washery, Coal handling plant, and coke oven plant

C) Steel plant.

D) Thermal power station

E) Fertilizer, Cement and Chemical industry.

A) Coal mines: -

It is mainly the coal bearing lower Gondwana region that predominates in the Damodar valley. They occupy 4,582sq. km means one tenth of the total area, and play a vital role in the economy of the region and also of the country. Coal reserve in this valley accounts for approximately 36.5% of the country's total coal reserve of 186 billion tones .According to Bannerjee *etal.*1998,[2] there are seven broadly identified coalfields in the Damodar that is .North Karanpura, South Karanpura, Ramgarh, West Bokaro, East Bokaro, Jharia, and Raniganj.

A) Coal washery; -

At present there are more than 15 coal washeries under operation in this coal belt which process 28.5 million tons of coal per annum. Another 3.00 million tons capacity at Kedla is awaiting for operation. Coal purification process consumes water in the range of 0.20 to 0.25m^3tons of raw coal input and the total demand of water is approximately 0.56 million^3. It has been estimated that 10 washeries having washing capacity of 17.04 million tons of coal consume 70 million gallons of water and produce 2 million tons of solid wastes and tailings per annum of which 7,000 tones find outlets to river course through effluent streams and remaining un disposed quantities through rain water. The huge quantum of washery effluent brings severe ecological changes in the river water, especially during the lean period. It imparts brownish black colour to the river water and then transparency comes down below 10 mm due to high concentration of dust present in the effluxion.

B) Steel plant: -

Country's three major steel plant that is – Bokaro Steel Limited (BSL), Bokaro, IISCO (Indian iron and steel company), Burnpur and DSP (Durgapur steel plant), Durgapur, producing 0.08 million tons of steel, draw mainly the water from the river Damodar. Water is among the most essential inputs for the iron and steel industry. Huge quantities of water are used in various processes-e.g.: cooling and purification of coke over gas that pollutes the water with tar oil, ammonia, phenol, cyanide, thiosulphate which ultimately find their way through the effluents. The total quantity of discharged effluents into the Damodar from the three steel plants BSL, DSP, and IISCO amounts to 273.13MLD.[2]

Coke oven plant: - In coke oven plants coal is burnt in the absence of air to increase fuel efficiency. The steam that is employed in the process produces effluents although in low volume with high levels of tar oil, ammonia and phenol.

C) Thermal Power Station: -

The Damodar River system hosts at present nine thermal power and three hydel power stations having the generation capacity of about 3,000 MW of electric power. Bokaro, Chandrapura, Durgapur, and recently established Mejia thermal power stations are run by the DVC while the Patratu Thermal Power Station is run by Jharkhand Government and the Loyabad, Bhowrah and Jamadoba are captive thermal power plants. These huge power generating plants solely depend on the water from the Damodar River system.

Effect of coal washery-

Release of the effluent generated during washing of coal became a major problem for coal industries due to presence of many toxic metals and other pollutants. These effluents are the main source of pollution in rivers and other water bodies. In a research work (CWE) collected from one of the washery plants situated in Dhanbad, India. CWE was found to be very toxic containing a large number of suspended particles and many heavy metals above than their permissible limits. Efficiency of *Pleurotus* for remediation of heavy metals was found to be highest in the 50% diluted effluent (57.2% Mn, 82.6% Zn, 98.0% Ni, 99.9% Cu, 99.3% Co, 99.1% Cr, 89.2% Fe and 35.6% Pb) followed by 25% diluted effluent (33.0% Mn, 55.1% Zn, 97.8% Ni, 99.7% Cu, 97% Co, 84.4% Cr, 87.1% Fe and 73.4% Pb) and raw effluent (23.3% Mn, 73.1% Zn, 78.7% Ni, 87.5% Cu, 59.3% Co, 64.6% Cr, 34.6% Fe and 11.3% Pb) respectively.[3]

Fish and Fishery: -

Various researchers have studied the Ichthyofaunal diversity of river Damodar. Notable among them are-Saha *et al* (2013). They have reported the occurrence of 46 fish species with the order *Cyprinidontiforms* dominating among others in Damodar river at Burdwan(West-Bengal).

How ever river Damodar has never been a potential fishery resource, neither there has been any well-defined commercial fishery especially in the upper and middle valley regions due to the typical physiography. This stretch experiences a devastating torrential flow during monsoon and in summer a narrow few centimetre deep meandering water strip and obviously could not harbour any commercial fishery.

In the upper most river stretch from Tori to Bhurkunda the species encountered were mostly minnows found in torrential waters represented by *Bariliussp; Puntiussp ; Noemachilussp ;Ambassissp; and Chela sp;* between Rajarappa and Tenughat mainly carps, found in mountain foothills namely *Lobeoboggut,L.dyocheilus, L.calbasu and Bariliussp;* were encountered. The cat fishes like *Mystusseenghala, M. aor, M. cavasius, Ompokbimaculatus* and *Clupisomagarua* were also encountered. (source- bulletin 79, The river Damodar and its environment)

As we know that the liver is the site of metabolism and it plays an important role in biochemical transformations of pollutants during the detoxification process. Due to toxicant exposure and accumulation, lesions and other histopathological alterations are common in the liver. [4]

Reddy *et al* (2010) and Reddy and Baghel (2012) [5] observed signs of degeneration (cytoplasmicand nuclear degeneration and nuclear vacuolation) and the focal necrosis in the liver parenchymaof fishes exposed to the industrial effluents.

As reported by Sujoy Patra,2008[6], Damodar River, once known as the “River of Sorrow” because of the flood havoc it used to cause, has now turned into a “River of Agony” due to the environmental degradation of the river resulting from the indiscriminate discharge of domestic, industrial and mining wastes from the basin. Going through the work of many researchers and their studies we came to know that the present physical and hydro biological condition of the river Damodar has deteriorated. The river has lost its natural flow pattern and except for monsoon, it is reduced into a drainage channel and receives discharges from numerous industries that flank along its banks. This impact is felt maximum in the river stretch between Tenughat and Panchet reservoirs.

Many surveys show that big industries located in this area are primarily responsible for degrading the ecosystem. In Spite of installing some effluent treatment plants, no significant improvement has taken place because more often they are found to be inoperative or not functioning. Moreover, the release of water from the reservoirs being very irregular, the flow rate varies frequently which upsets the normal life cycle of the biotic communities.

The most pronounced human activity that threatens the existence of the river is the indiscriminate dumping of the solid wastes mostly from open cast mining and other industries. The dumps along the banks are narrowing the river course and as well raising river bedtogether with drifted sand and fly ash.

The product of coal washeries have made the river polluted, full of toxicants, not useful for fishery, not for drinking and other works.

Hence, in the present study it is proposed to evaluate the effect of coal washeries on the biodiversity specifically on the fish species which abound in such an environment. Our present study area, Bokaro district is situated in state Jharkhand which is situated in the eastern part of our country India. It is the homeland of India's largest steel plant Bokaro steel plant and several other small-scale industries like cement factory, stone crusher, bricks factory etc.

2. REVIEW OF LITERATURE:

Apurba Ratan Ghosh and Ruma Banerjee, 2012[7] have done research on the qualitative evaluation of Damodar River flowing over coal mines and industrial areas found that the water quality of the river was influenced by the seasons and the coal mines contribute to increase Electrical conductivity (EC), chloride and sulphate concentration in the water.

Banerjee *et.al* (2010) [8], have observed in their research work that the changes in the physicochemical parameters of water such as PH, Temperature, water hardness, dissolved

oxygen, presence of chelating materials. Salinity, rainfall, climate changes etc, all affect the presence of heavy metals in the aquatic environment.

Banerjee and Gupta (2010),[9] studied the seasonal status of irrigation water suitability of river Damodar in West Bengal, India. According to them, Dissolved oxygen, a barometer of the ecological health of the stream, is one of the most important parameters for protecting aquatic life.

Chakaraborty, Rina (2011),[10] Through this study it was revealed that the well forested catchments of rivers always provide a healthy faunal diversity to nature for the betterment of mankind. Thus, no developmental activities should be entertained at the cost of nature which is practically impossible to regain.

Davies *et al*, (2009) [11], have conducted a research work on seasonal abundance and distribution of plankton. The study reflected that the stream is under stress due to the high phosphate concentration and is pertinent to note that only treated effluents must be discharged into the stream. They recommended that waste facilities should be provided for the surrounding dwellers by the government and laundry activity should be discouraged by the government.

Ghose M.K. (1999), [12] studied that the effluents from coal washeries cause serious pollution problems to surface waters. For the removal of suspended solids from the coal washery effluents the aid of a synthetic flocculant was found to be very effective in terms of settling rate, retention time and cost. For sustainable water supplies to the coal washeries the methodology developed was found to be very effective and may be applicable to other washeries.

Hassan *et al.* (2015) [13], reported the biochemical changes in muscles and liver of Kosher Fish (Epinephelus summana) in relation to helminth infection. In the study, carried out in Jeddah, Saudi Arabia, a total of 102 E. summana were collected randomly from the Red Sea, Jeddah coast during the period of March to September 2014. Helminthes parasites were detected in the liver, intestine and stomach of the infected fish. Total protein and carbohydrates in muscles and liver was also quantified using spectrophotometer. Their results showed that the total muscle protein of fish and total carbohydrates of both muscles and liver decreased significantly in

infected fish. They reported that intestinal trematodes had higher effect on the muscle protein rather than stomach trematodes, whereas intestinal nematodes had a low effect on muscle protein than the stomach nematodes. They attributed this decrease might be due to the weakened ability of the intestinal absorption by the infected fish as the digested food is consumed by the parasite.

Jayakumar *et al*. (2009), [14] have conducted research on the alteration in physico-chemical parameters leading to eutrophication has become a widely recognized problem of water quality deterioration.

J.George *et al. (*2010), [15] conducted research on the Impact of coal industries on the quality of Damodar river water .It shows that the quantity of the dissolved and suspended solids ,hardness, COD . CBCs are higher due to the disposal of the wastes from coal washing plants, coke ovens, cement and other industries, but well within the permissible limit which is probably attributable to the high carrying capacity of the river.

Khan *et al* (2013) [16] The studies done by them in the mining areas of Singrauli, Madhya Pradesh clearly demonstrated that the water quality in close vicinity to the mining area is unsuitable for domestic purpose because of waste dumps and sewage discharge from industries when compared with the standards proposed by WHO. According to them the consumption of water around the mining and industrial area may cause health hazards to the local residents.

Khatoon, N. Rehman, M, and Khan A. F (2013),[17] have worked on the seasonal variation of physico-chemical parameters of river Ganga at Kanpur. Various observations were done. After the research, the observed water quality suggests that the water could not be used for drinking purposes. It could only be used for irrigation

Kumar *et.al*. (2015),[18] studied the distribution of protein, lipid and carbohydrates in muscle and liver of infected and normal *Upeneus vittatus*, a marine species found in the coast of Bay of Bengal, Visakhapatnam. It is observed in the study that there were significant differences in the Lipid content of infected and normal fish. Lipid levels were observed to be more in the fish with infection as compared to the non-infected fish.

Morina *et al* (2013) [19] According to them fish liver histopathology is a good bioindicator and can be used for detection of chemical pollution in fish. The work of **Dutta Munshi *et al* (1993) [20]**, also suggests that the presence of certain chemicals e.g. malathion induces histopathological changes in the liver of freshwater catfish.

Olimax and Sikorska, (1975) [21] The Physico-chemical characters can be changed by inflow of different pollutants and nutrients through different sources like sewage, industrial effluents/agricultural runoff etc. Which can be subjected by several investigations

Okoye *et.al*. (2014), [22] studied the prevalence, mean intensity, abundance and seasonality of parasites of fish from natural freshwater tropical Agulu Lake (natural, freshwater tropical lake), in southeast Nigeria. In the study, a total of 1191 fish specimens belonging to four families (Cichlidae, Bagridae, Hepsetidae and Channidae) were collected and examined for the parasites. The study reported 11 species of parasites comprising metacercariae of three digenetic trematodes, one cestode, five nematodes and two acanthocephalans.

Pilla *et al.* (2014),[23] studied the biochemical compositions of muscle and liver of normal and infected fish of Lutjanus johni off Visakhapatnam Coast. Proteins, lipids and carbohydrates in muscle and liver tissues were studied with respect to different seasons. The study reported that the level of Lipids were elevated, (10.27±0.58mg/g,), while protein content declined 74.60mg/g, (±2.61) in the infected liver tissues as compared to the normal fish (78.29±1.86mg/g). Some fluctuations were also observed by these workers in total carbohydrate content in the infected fishes.

Rita, Kumar, Solanki & Kumar, (2012), [24] have also worked on the use of rivers not from now but since old times rivers have also been used for cleaning and also for disposable purposes.

Tiwary and Abhishek, (2005) [25], have observed that the physico-chemical analysis of Damodar River in and around coal washeries show that parameters such as TDS, TSS, BOD, and COD are high which indicates that suspended and dissolved solids from washeries are contaminating the river water.

3. Problem to be investigated

In the proposed thesis the investigator aims to investigate the following problems:

1. To study the effect of coal washeries on the water of river Damodar.
2. To know the effect of coal washeries on the liver of fish found in the river.

4. Objectives of the research

- To analyse the water of river Damodar at the confluence points of various coal washeries.
- Microscopic study will be done to know the histo pathological changes in the liver of the fish due to the effect of coal washery.

5. Hypotheses:

Through this research the investigator will try to know the correlation between the consummability of various fish species found in Damodar River in the washery area and the pollution load of the river water. It may be hypothesised that due to abnormal changes in the physico-chemical characteristics of river water the fish fauna is adversely affected and it may not be suitable for human consumption.

6. Research tools and methodology:

- Fresh food fish commonly available in the river Damodar will be collected and transported to the laboratory. Healthy live specimens will be allowed to acclimatize to the laboratory condition.
- Physico -chemical characteristics of ground water of the sample sites and Damodar River water will be estimated by adopting standard procedure. (APHA 1998).[26]
- Gross morphological and histological changes in the liver of the fish species will be studied after section cutting and staining.
- Various experiment related tools such as microtome, should be in good condition
- Various statistical tools like Correlation, Standard Deviation, ANOVA will be used for the validation of the results obtained.

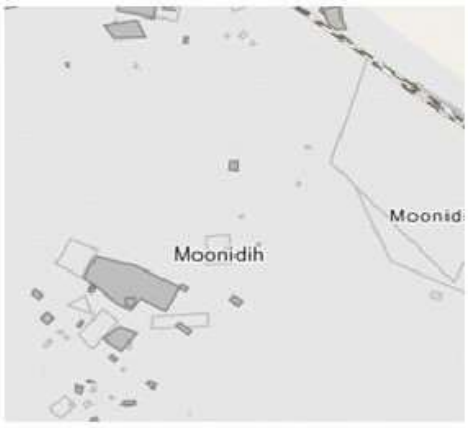
1) Study Area

Four sampling sites will be selected on the Damodar River between the districts of Bokaro and Dhanbad. The latitude and longitude of the study area is (2215'to 2430'N latitude and 8430'to 8815' E longitude). The study areas are:

- Site -1: Bhojudih. (Chas-Bokaro)
- Site -2: Moonidih (Dhanbad)
- Site-3: Dugda (Bokaro)
- Site-4: Sudamdih (Dhanbad)

Map 1 – sampling sites

MAP 2 - MOONIDIH



MAP 3 - BHOJUDIH

2) Collection of samples-Samples will be collected three times in one calendar year *i.e.* triennially from the above-mentioned area. Sampling will be done three times according to the following seasons: -

(a) Monsoon-August, September, October and November

(b) Winter- December, January, February and March

(c) Summer- April, May, June and July.

(i) For water quality analysis, sampling will be done monthly. Water will be collected from all the four sampling sites in clean PVC containers of 2 litres and transported to the laboratory for analysis. The laboratories where samples for physico – chemical parameter will be tested are – Bokaro steel City College (Zoology lab) and P.G Department of Zoology, BBMKU- Dhanbad

(ii) For the histopathological examination of fish, the fishes will be caught from all the four sampling sites of Damodar River with the help of local fishermen and residents. The fish will be dissected on the site itself and the liver will be taken out and fixed in 10% formalin. After that itwill be taken to the laboratory for further examination.

Time period-The research work will be done for two consecutive years starting from August 2020 to August 2022 to get the exact result.

3) Lab Analysis-Analysis of various physico-chemical parameters will be performed as per the guidelines of APHA-1998. [26]

Testing instruments and procedure-

a) For physical test

1. pH: By digital pH meter.
2. Temperature: By digital thermometer.
3. Turbidity: By Secchi disc technique.
4. Nitrate: Nitrate Electrode method/Phenol Disulphonic Acid (PDA)Method (APHA,1998).
5. Phosphate: Stannous Chloride Method (APHA 1998)
6. Sulphate: Gravimetric Method (APHA 1998).

b) For chemical test

1. D.O(Dissolved Oxygen): Winkler's Method or Iodometric Method according to APHA, 1998.
2. B.O.D (Biochemical oxygen demand): Dilution standard method (APHA,1998).
3. C.O.D(Chemical oxygen demand): standard method according to APHA,1998.
4. T.D.S(Total dissolved solid): standard methods according to APHA,1998.

4) ANALYSIS OF WATER PROFILE FOR PHYSICO-CHEMICAL TEST

For collection of sample analysis of various physico – chemical parameters will be performed as per the guidelines of APHA-1998. Physico – chemical parameters will be – Total Suspended Solids (TSS), Total Dissolved Solids (TDS), pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), nitrates, phosphates, sulphates, etc.

Physico-chemical parameters will be analysed in this research are as follows –

1. Dissolved Oxygen (DO) – DO will be measured following iodometric method (APHA, 1998). The iodometric method is a titrimetric procedure based on the oxidizing property of dissolved oxygen.
2. Biochemical Oxygen Demand (BOD) – The Biochemical Oxygen Demand (BOD) determination is an important test in which standardized laboratory procedures will be used to determine the relative oxygen requirements of Damodar River.
3. Chemical Oxygen Demand (COD) – COD is a measure of the amount of oxygen required to oxidize the organic matter. COD will be measured by Open Reflex Method (APHA, 1998). The method involves reflex of a sample in a strong acid with an excess of potassium dichromate.
4. Total Dissolved Solids (TDS) – Total solids include Total Suspended Solids (TSS), the portion of total solids retained by a filter; and Total Dissolved Solids (TDS), the portion that passes through the filter. TDS and TSS will be determined following the methods described in 'Standard Methods for the Examination of Water and Wastewater' (APHA, 1998). A well-mixed sample will be filtered through a standard glass fibre filter, and the filtrate will be evaporated to dryness in a weighed dish and dried to constant weight at 180^0C. The increase in dish weight will represent the weight of Total dissolved solids.
5. Nitrates (NO_3) – Nitrates will be estimated by Phenol Disulphuric acid to form a nitro derivative, which in an alkaline medium develops yellow colour.
6. Phosphates (PO_4) – Though phosphorus in a limited concentration in water bodies is necessary for aquatic life, higher concentrations of PO_4 are indicative of pollution as phosphate promotes eutrophication. Phosphates in water react with ammonium molybdate and form a complex

molybdophosphoric acid, which gets reduced to a complex of blue colour in the presence of stannous chloride.

7. Sulphate (SO_4) – Sulphate will be analysed in water samples following Gravimetric method (APHA, 1998).

Methodology Of showing the changes in the liver of the fish:

Histopathology is one of the most effective tools in diagnosing tissue abnormalities. (K.L. Mukherjee, 2005) [27]. The process of histological staining takes five key stages which involve- fixation, processing, embedding, sectioning and staining Titford,2009.[28]

The steps can be divided into-

1. Preparation of tissue which includes fixation and decalcification.

2. Processing of tissue which includes dehydrating, clearing, and embedding.

3. Preparation of sections which includes the process of microtomy, attaching sections to slide and removal of pigments and precipitates.

4. staining and mounting procedure: de waxing, hydrating, staining, dehydrating and mounting.

Fixation- The fixation phase retains the chemical composition of the tissues, hardens the cells or tissues for sectioning and delays degradation [28]. For fixation 10ml of 10%formalin will bedissolved in 90ml water and 0.9 gm NaCl will be added.

Tissue processing- Water is removed from fixed tissue known as Dehydration. Ethyl alcohol or ethanol is used as dehydrant [29]. Embedding is done with paraffin wax. Xylene is used as a clearing agent. After clearing tissues will be transferred to a bath of molten paraffin wax in an embedding oven for infiltration and impregnation.

Preparation of sections- The embedded tissue will be taken for section cutting. It involves the techniques of microtomy which gives thin ribbon-like sections of the tissue for microscopic examination of the internal structure. It is important that microtome knife should be in good condition.

Technique of section cutting Trimming of paraffin block will be done. After that slides will be prepared.

Staining- First of all the section will be dried in the oven and deparaffinized with xylene. After washing it will be stained with haematoxylin for 3-5 minutes. Haematoxylin is a basic dye which gives the nuclei a bluish colour Black,2012.[30] It will be washed again in running water. After

that slides will be dipped in and out of 5% hydrochloric acid. After washing in distilled water for a short while it will be dipped in dilute ammonia water also.
It will be placed in 95%alcohol,75% alcohol for 30-60 sec. Then cleared in xylene.
The excess stain will be allowed to drain, and the slides will be mounted on D.P.X with cover slip.

5) Statistical Analysis

All the data obtained during the course of study will be statistically verified considering Correlation between the various physico chemical parameters, Standard Deviation and ANOVA.

7. Significance of proposed work

This research work will help in the development of socio-economic standards of people of Bokaro district whose livelihood is based on the fishery and its products. Coal washeries effluents pollute the aquatic bodies and is harmful for the survival of the fishes. By this pollution effect the society is also affected by and large. Through this present study the toxicants will be identified, and the pollution load will be minimized through the various steps suggested after the investigation is complete.

8. Future work:

The future prospect of the proposed research work would be to solve the problem of water pollution. Water may be used for drinking, Cooking, etc. It will also help to save the life of fishes found there.

9. Research limitation:

It is neither possible nor desirable for any researcher to study a set of phenomena at all levels from all possible angles in a time bound research like the present one. Various constraints related to the content of the research and linked resources like Firm and finance compel every investigator to delimit his/her endeavour, in respect of scope and level of which the study is

ultimately conducted. The present study is no exception also. This research work will be carried out in a short span of time and various aspects like study area, sample tools, time, etc. might pose limitations for the researcher.

10. Tentative Chapterization:

Chapter1: Introduction

This chapter will give introduction to the work carried out and its application in the various areas of science. The advancement made in the related field of research will also be presented here. An outline of the thesis will be presented.

Chapter 2: Literature Review

This chapter will give a comprehensive summary of previous research papers by various researchers around the globe on the topic related to aquatic pollution and its effect on fish morphology and pathology.

Chapter 3: Water Quality Analysis

In this chapter the complete analysis of various physico-chemical parameters of Damodar River will be presented.

Chapter4: Methodology Of showing the changes in the liver of the fish:

This chapter will deal with various methods used to find out the changes in the liver of the fish as a result of aquatic pollution.

Chapter5: Statistical Analysis

All the data obtained during the course of study will be statistically verified considering Correlation between the various physico chemical parameters, Standard Deviation and ANOVA.

Chapter 6: Conclusion-Through this research work we will do the remedial measures to save the river Damodar, the life of fish species and the life of people who are surviving on the aquatic animals of the river.

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